The dynamics and genetic adaptation to salt stress in experimental evolution of *Desulfovibrio vugaris* Hildenborough

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ESPP Environmental Stress Pathway Project

DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT — OFFICE OF SCIENCE U.S. DEPARTMENT OF ENERGY

VIMSS Virtual Institute for Microbial Stress and Survival | University of Missouri-Columbia | University of Washington | Montana State University | Oak Ridge National Laboratory | Sandia National Laboratories | OU | Berkeley University of California | 75 Science

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ABSTRACT

One of the greatest challenges in biology is to understand the interaction between genotype and environment to determine the fitness of an organism. With the recent advances in genome sequencing and high-throughput genomic technologies, now it is possible to link sub-cellular molecular/metabolic processes with the population-level processes, functions and evolution. Sulfate reducing bacteria *Desulfovibrio vugaris* Hildenborough (DvH) is an ideal model environmental organism to address such fundamental questions. In this study, the long-term evolutionary responses, diversifications and adaptation of DvH to salt stress were investigated by mimicking the stress condition in the lab culture. The phenotype of the cell lines with higher salt concentration were tested periodically. The results demonstrated that the adaptation to salt stress is a dynamical process. The enhanced salt tolerance to higher salt (LS4D + 250 mM NaCl) of stressed lines was observed at 300 generations and it became more obvious with the increase of generations. The de-adaptation experiment on 500, 1000 and 1200 generation cell lines not only provided evidence that the phenotype was due to the genetic change, but also demonstrated that the genetic mutation became stable at 1000 generation. To further decipher the genetic mystery in behind, the gene expression profile of the 1000 generation were examined by DvH whole genome microarray. Some poly-cistronic operons such as hmcF-E-D-C-B-A, rrf2-rrf1, LysA-2-LysX and DVU3290-3291-3292 (glutamate synthase) were significantly up-regulated in stressed lines. Next, whole genome sequencing on selected colony will be performed to identify the beneficial genetic mutation and more colonies will be checked to confirm

MATERIALS AND METHODS

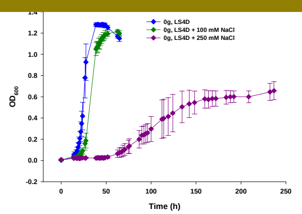
Bacteria strain: Single colony-based liquid culture was obtained from the original *D. vulgaris* Hildenborough stock. Six lines each were used for control and treatment respectively.

Medium and culture condition: LS4D was used as standard medium for the control. Medium for salt stress treatment was LS4D +100 mM NaCl. Cells were kept at 37°C and transferred every 48 hrs.

Handling of the samples: At some selected points, for example, every 100 generations, the glycerol stocks were archived and a variety of genetic, molecular, physiological, and genomic analyses were conducted to determine their evolution/adaptation to environmental stresses.

Microarray analysis: 70mer oligonucleotide arrays for *D. vulgaris* Hildenborough that containing all ORFs (He et al., 2006) were used in this study. Total cellular RNA was isolated using TRIzol (Invitrogen) and RNeasy mini column and labeled with Cy5 dye. Genomic DNA was isolated from *D. vulgaris* Hildenborough as described previously (Zhou et al., 1996) and labeled with Cy3 dye. The labeled cDNA and genomic DNA were co-hybridized to the array. Microarray data were processed as described before (Chhabra et al., 2006; Mukhopadhyay et al., 2006).

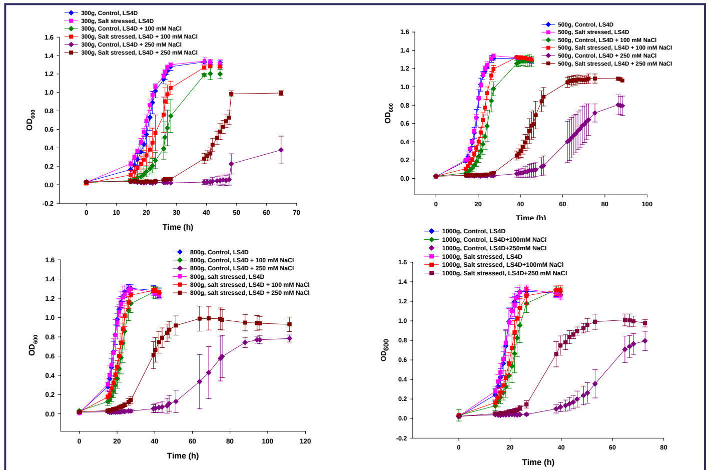
RESULTS



Effect of salt on the growth of for the original DvH strain

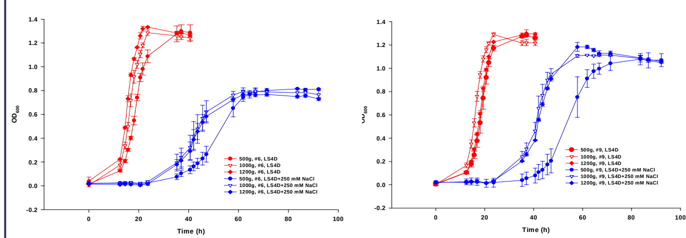
With 100 mM of NaCl in the medium, the growth of DvH was delayed for a few hrs; the growth rate and final biomass were not affected;

With 250 mM of NaCl in the medium, there was a very long lag phase and the final biomass was only half of the control.


300g, Control, LS4D
300g, Salt stressed, LS4D
300g, Control, LS4D + 100 mM NaCl
300g, Salt stressed, LS4D + 100 mM NaCl
300g, Control, LS4D + 250 mM NaCl
300g, Salt stressed, LS4D + 250 mM NaCl
500g, Control, LS4D
500g, Salt stressed, LS4D
500g, Control, LS4D + 100 mM NaCl
500g, Salt stressed, LS4D + 100 mM NaCl
500g, Control, LS4D + 250 mM NaCl
500g, Salt stressed, LS4D + 250 mM NaCl
800g, Control, LS4D
800g, Control, LS4D + 100 mM NaCl
800g, Control, LS4D + 250 mM NaCl
800g, salt stressed, LS4D
800g, salt stressed, LS4D + 100 mM NaCl
800g, salt stressed, LS4D + 250 mM NaCl
1000g, Control, LS4D
1000g, Control, LS4D+100mM NaCl
1000g, Control, LS4D+250mM NaCl
1000g, Salt stressed, LS4D
1000g, Salt stressed, LS4D+100mM NaCl
1000g, Salt stressedl, LS4D+250 mM NaCl
OD600
Time (h)

The adaptation to salt stress was obtained during the long-term culture under laboratory controlled conditions

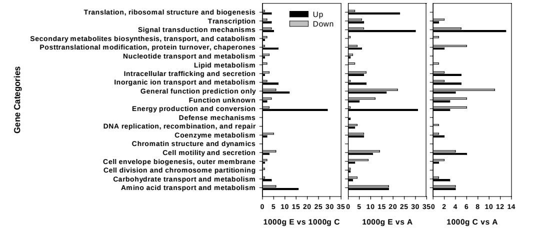
The growth of 300, 500, 800 and 1000 generation samples was tested on LS4D, LS4D +100 mM NaCl and LS4D + 250 mM NaCl. On high salt medium LS4D + 250 mM NaCl, the lag phase of evolved lines under mild salt stress (100 mM NaCl) became shorter with the increase of generation number; more biomass was produced compared to the ancestor.



The enhanced salt tolerance is due to genetic changes instead of physiological adaptation

The de-adaptation experiment was done with 500g, 1000g and 1200g cell lines evolved with mild salt stress. All the cell lines from these generations were grown on standard medium LS4D for about 50 generations before the phenotype test on higher salt medium (LS4D +250 mM NaCl). #6 is a control line grown on LS4D medium; #9 is a de-adaptive line evolved on LS4D + 100 mM NaCl. Three out of 6 evolved lines under salt stress and 2 out of 6 control lines showed stable phenotype with the tested 1000g and 1200g samples. In addition, the evolved lines on salt stress demonstrated better salt tolerance than the control lines.

Gene expression profiles at 1000 generation


Translation, ribosomal structure and biogenesis
Transcription
Signal transduction mechanisms
Secondary metabolites biosynthesis, transport, and catabolism
Posttranslational modification, protein turnover, chaperones
Nucleotide transport and metabolism
Lipid metabolism
Intracellular trafficking and secretion
Inorganic ion transport and metabolism
General function prediction only
Function unknown
Energy production and conversion
Defense mechanisms
DNA replication, recombination, and repair
Coenzyme metabolism
Chromatin structure and dynamics
Cell motility and secretion
Cell envelope biogenesis, outer membrane
Cell division and chromosome partitioning
Carbohydrate transport and metabolism
Amino acid transport and metabolism
Gene Categories
Up
Down
1000g E vs 1000g C
1000g E vs A
1000g C vs A

Selected operons with altered expression level

Gene name	Description	1000g C vs A	1000g E vs A	1000g E vs 1000g C
DVU3290	conserved domain protein, glutamate synthase	-0.16 (-0.21)	0.91 (1.10)	0.98 (1.01)
DVU3291	glutamate synthase, iron-sulfur cluster-binding subunit, putative	0.06 (0.06)	2.51 (2.34)	2.42 (2.61)
DVU3292	pyridine nucleotide-disulfide oxidoreductase	-0.08 (-0.14)	1.40 (2.11)	1.40 (2.07)
hmcF	HmcF, 52.7 kd protein in hmc operon	0.18 (0.19)	1.96 (2.20)	1.78 (2.42)
hmcE	HmcE, 25.3 kd protein in hmc operon	0.65 (0.93)	2.45 (3.88)	1.81 (2.93)
hmcD	HmcD, 5.8 kd protein in hmc operon	-0.22 (-0.26)	2.21 (3.77)	2.38 (2.92)
hmcC	HmcC, 43.2 kd protein in hmc operon	0.14 (0.25)	1.24 (2.27)	1.11 (1.89)
hmcB	40.1 kd protein in hmc operon (HmcB)	-0.27 (-0.37)	0.27 (0.35)	0.55 (0.74)
hmcA	HmcA; high-molecular-weight cytochrome c	-0.64 (-0.60)	1.03 (1.15)	1.58 (1.84)
lysA-2	diaminopimelate decarboxylase	-0.33 (-0.40)	1.74 (2.41)	2.00 (2.46)
lysX	predicted transcriptional regulator for lysine biosynthesis and transport	0.05 (0.05)	0.89 (1.02)	0.83 (1.10)
rrf2	Rrf2 protein	-0.15 (-0.16)	1.35 (1.71)	1.48 (1.87)
rrf1	Rrf1 protein	0.09 (0.08)	1.83 (2.01)	1.72 (2.27)

Gene name	Description	1000g C vs A	1000g E vs A	1000g E vs 1000g C
DVU2405	alcohol dehydrogenase, iron-containing	-1.41 (-1.15)	0.18 (0.32)	1.63 (1.32)
htpG	heat shock protein HtpG	-0.80 (-1.52)	0.74 (1.21)	1.53 (2.34)
DVU1169	methyl-accepting chemotaxis protein	-1.64 (-1.72)	-0.02 (-0.02)	1.42 (1.47)
DVU0845	hypothetical protein	-0.13 (-0.19)	1.19 (2.11)	1.37 (1.89)
DVU2618	hypothetical protein	-0.34 (-0.52)	1.06 (1.23)	1.36 (1.50)
DVU0671	conserved hypothetical protein	0.44 (0.83)	1.71 (3.04)	1.28 (2.26)
DVU0094	methyl-accepting chemotaxis protein	0.50 (0.75)	1.73 (2.68)	1.26 (2.14)
ApsB	adenylylsulphate reductase, beta subunit	-0.16 (-0.19)	0.47 (0.86)	0.64 (0.75)
ApsA	adenylyl-sulphate reductase, alpha subunit	-0.10 (-0.14)	0.83 (1.48)	0.94 (1.32)
QmoA	oxidoreductase	-0.01 (-0.01)	0.67 (1.14)	0.73 (0.93)
QmoB	oxidoreductase	0.02 (0.03)	1.14 (2.17)	1.13 (1.86)
QmoC	oxidoreductase	-0.20 (-0.26)	0.97 (1.39)	1.17 (1.77)
NA	hypothetical protein	-0.17 (-0.27)	0.61 (1.08)	0.77 (1.18)

E: evolved lines under salt; C: control lines with standard medium; A: ancestor lines;
Gene categories are according to the COG functions.
In the gene expression of selected operons, pink means the increase of gene expression and light blue means the decrease of gene expression. The number is the $\log_2 R$ and the Z score is shown in the parentheses.

SUMMARY

- The adaptation of DvH to salt stress is a dynamical process.
- The growth difference between control and salt stressed cell lines were observed at 300 generations and getting more obvious with an increase in the generation number; salt-stressed lines were more tolerant to the increased salt concentration (250 mM NaCl).
- The de-adaptation experiment suggested that there were genetic bases for the phenotypic changes along with the generations.
- Microarray data showed that "energy production and conversion" and "amino acid transport and metabolism" were the gene categories with the most number of up-regulated genes.

FUTURE WORK

- Genome sequencing of single colony from evolved lines with stable phenotype to discover the possible beneficial mutations and confirm the mutations by traditional Sanger sequencing of the PCR amplified fragment;
- Metabolite assays and proteomics analysis;
- Gene complementation to confirm gene functions;
- Study of the possible phenotypic changes with different stressors.

ACKNOWLEDGEMENT

ESPP is part of the Virtual Institute for Microbial Stress and Survival supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics:GTL Program through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.